Yale Scientists "See" Basis of Antibiotic Resistance

Using x-ray crystallography, researchers at Yale have "seen" the structural basis for antibiotic resistance to common pathogenic bacteria, facilitating design of a new class of antibiotic drugs, according to an article in the April, 2005 issue of *Cell*.

In recent years, common disease–causing bacteria have increasingly become resistant to antibiotics, such as erythromycin and azithromycin. Although the macrolide antibiotics in this group are structurally different, all work by inhibiting the protein synthesis of bacteria, but not of humans. They bind tightly to an RNA site on the bacterial ribosomes, the cellular machinery that makes protein, but not to the human ribosomes. Bacteria can become resistant to antibiotics in several different ways. When bacteria mutate to become resistant to one of these antibiotics, they usually are resistant to all antibiotics in the group.

Authors (from left) Daqi Tu, Peter B. Moore,Thomas A. Steitz, and Gregor Blaha

Studies led by Sterling Professors Thomas A. Steitz and Peter B. Moore in the Departments of Molecular Biophysics and Biochemistry, and Chemistry at Yale University illuminate one of the ways that bacteria can become resistant to macrolide antibiotics.

"A major health concern of antibiotic resistance is that two million people every year get infections in hospital facilities and 90,000 per year die from them," said Steitz. "Macrolide-resistant *Staphylococcus aureus* is the most common of these infections."

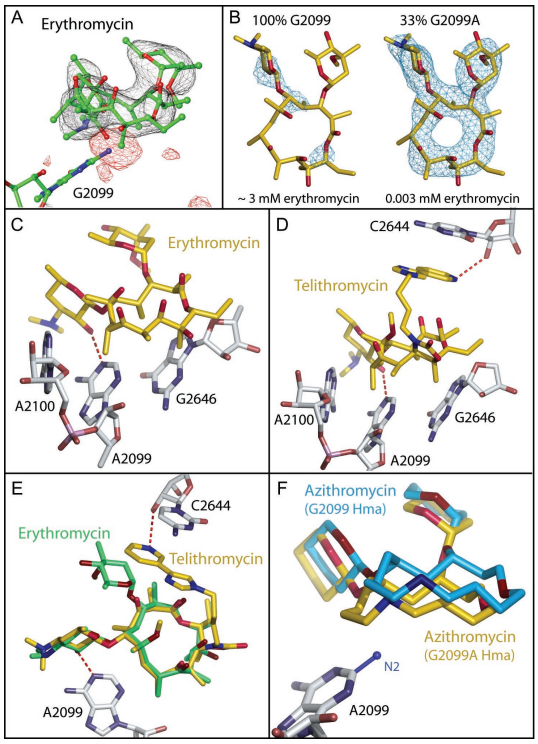
Some of the clinically important bacteria are resistant because of mutation of a single nucleotide base, from an A to a G, in the site where macrolide antibiotics bind to the ribosome. The Yale group was able to "see" structural alterations when antibiotics were bound to ribosomes with different sensitivity to the drugs because of mutation. They can now explain why that mutation has the effect that it does. "The mutant G has an amino group that pokes into the center of the macrolide ring, causing it to back off the ribosome by an Angstrom or so," said Steitz. The change of that one base in the ribosomal RNA reduced the ability of the antibiotic to bind by a factor of 10,000.

Mutation of this type happens naturally, but rarely — only one in 100,000 to one in 10,000,000 bacterial mutations will cause this kind of resistance. However, each bacterium can divide as often as every 20 minutes, allowing one with a resistant mutation to rapidly cause a dangerous infection.

Daqi Tu, a student, and Gregor Blaha, a postdoctoral fellow in molecular biophysics and biochemistry and associate of the Howard Hughes Medical Institute, are co-authors on the study. Funding for this research was obtained from the National Institutes of Health and the Agouron Institute.

For more information, see: D. Tu, G. Blaha, P.B. Moore, and T.A. Steitz, "Structures of MLS_BK Antibiotics Bound to Mutated Large Ribosomal Subunits Provide a Structural Explanation for Resistance," Cell, ***121(2)****, 257-270 (2005).*

— Janet Rettig Emanuel, Yale University



Interactions of macrolides with G2099A large ribosomal subunits. (A) A (Fo(mutant + drug) – Fo(wild type – drug)) difference map calculated using wild-type phases shows positive 4 σ density (black) for erythromycin, and a negative 4 σ peak (red) at N2 of G2099. (B) No convincing difference density is observed when erythromycin is soaked into wild type H. marismortui 50S crystal with ~ 3 mM concentration (left), but when it is soaked into 50S crystals containing 33% G2099A mutants, clear density is observed when the drug concentration is 3 μM (right). (C) Erythromycin binds in the hydrophobic pocket formed by residue A2100 (A2059), A2099 (A2058) and G2646 (C2611), with its desosamine nitrogen hydrogen bonded to A2099N1. (D) Telithromycin binds in the hydrophobic pocket formed by residue A2100, A2099 and G2646 the way erythromycin does, with its alkyl-aryl extension making an additional stacking interaction with the base of C2644 (U2609), and a hydrogen bond to the 2'OH of C2644 (U2609). (E) The lactone rings of erythromycin and telithromycin bound to Hma ribosomes are perfectly superimposable. (F) Comparison of azithromycin bound to G2099 (blue) and G2099A (yellow) ribosomes. The N2 of a G was modeled onto residue A2099. The two structures were aligned by least squares superimposition of ribosomal RNA phosphorus atoms.